EVIDENCE OF DAILY AND HOURLY GROWTH IN PILCHARD LARVAE BASED ON OTOLITH GROWTH INCREMENTS, *SARDINA PILCHARDUS* (WALBAUM, 1792)

by

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ABSTRACT. – The method outlined in the present study provides an easy mean for corroborating the daily nature of otolith increments of field-captured larvae. The exact hour of the formation of the two parts of each daily unit (organic and inorganic) in pilchard larvae was established. The protein-rich portion of the daily increment is deposited during the first hours of darkness. The initiation of a new daily growth increment began around 00.30 h (local time).

RESUME. – La méthode que nous avons développée dans ce travail permet la détermination, chez les larves de poissons capturées en mer, de la nature journalière des anneaux, observés sur les otolithes. L'heure exacte de la formation des deux parties de chaque anneau journalier (organique et inorganique) a été établie pour les larves de sardine. La fraction riche en protéines de chaque anneau est déposée pendant les premières heures de nuit. Un nouveau dépôt journalier débute aux environs de 00.30 h (heure locale).

Key-words : Sardina pilchardus, Pisces, Daily Growth, Otolith reading

Fish otoliths grow by addition of layers of material differing in the relative amount of protein (otolin) and calcium carbonate in the aragonite form (Degens *et al.*, 1969; Pannella, 1971). This results in growth units or increments composed of an inner light band and an outer dark band that can be deposited on a daily basis. These two parts visible under the optical microscope correspond to two different production rates of fibrous material. During fast deposition rate the production of organic fibers is high, but calcification is even higher. During slow deposition fewer organic fibers are produced, but the ratio is in favor of the organic portion since calcification is almost nil,(Pannella, 1971).

The exact hour when the different portions of each daily growth unit are formed (refered hereafter as inorganic for the fast deposition zone and organic for the slow deposition zone) have received little attention from the authors who deal with daily growth studies. Brothers (1981) refers that the protein-rich portion of simple daily growth units is formed at night during the period of falling water temperatures without presenting any supporting data. Brothers and McFarland (1981) mentioned that the observation of the margin of otolith constitute a mean

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for corroborating the daily nature of growth increments. Tanaka *et al.* (1981) determined the exact hour when a new increment is formed in the otolith of *Tilapia nilotica* under experimental conditions.

Several environmental factors have been related to the formation, deposition and development of daily growth increments. Taubert and Coble (1977) were the first authors to state, based on experimental work, that a diurnal clock entrained by a 24-h light/dark cycle was responsible for the formation of daily rings. Brothers (1981) mentioned that temperature was the predominant factor in determining the formation, deposition and thickness of growth increments, with food and light having subordinate roles. The existence of an endogenous rhythmicity unaffected by photoperiod or temperature fluctuations was suggested by Campana and Neilson (1982). Neilson and Geen (1982) refer that feeding frequency influence both growth increment number and width, whereas photoperiod and temperature affect only increment width. There is reason to believe that no one factor alone is responsible for the production of otolith growth increments, instead it seems that an endogenous rhythm more or less affected by light, temperature and food might be the possible explanation. The relative influence of the refered environmental factors seem also to vary among fish species due mostly to the different ecological conditions.

The objectives of this study are: (i) to present evidence of daily ring formation in field-captured pilchard larvae; (ii) to determine the timing of the formation of the organic and inorganic portions of each daily growth increment; (iii) to relate the formation of daily units with several environmental factors in sea-caught larvae.

On a previous paper (Ré, 1983a), we suggested the existence of daily growth increments in the otoliths of pilchard larvae and that these might be used to age field-captured specimens. More recently (Ré, 1983b) the growth rates of sardine larvae and the thickness of daily units were found to vary in relation to the period of the year. This variation is probably related to differences in food availability and different feeding conditions.

MATERIAL AND METHODS.

Sardine larvae were collected on a fixed station (38°30' lat. N, 9°45' long. W) on the central part of the portuguese coast. Samples were made every hour during a period of 20 hours (15:30 to 10:30 local time) in December 9, 1982. We used a I meter ring trawl of 0.500 mm mesh aperture that was hauled on an oblique tow during 10 minutes at a constant speed of 2 knots. Tows were made to a maximum depth of 25 metres, the bottom at the station being 40 metres. The average volume of water strained was 208.3 m^3 ($s = 60.4$). The average number of pilchard larvae sampled was 0.36 larvae/m^3 ($s = 0.22$).

All samples were fixed immediatly after collection with 5 % formalin buffered with borax (pH 8.5 - 9).

The methods used for obtaining, mounting and observing the sagittae were mentioned in a previous paper (Ré, 1983a). It was not necessary to ground and polish the otoliths as sufficient light was transmited through the relatively thin sagitta for examination. This technique can be used with good results when examining the otoliths of pilchard larvae under the optical microscope up to a chronological age of about 50 days.

Measurements were made on otoliths dissected from larvae sampled at different hours of the day. In order to establish the timing of the formation of each daily growth increment, several otolith microphotographs were made. The relative width of the two last growth units was measured on the photographic negatives with a stereomicroscope and a calibrated micrometer eyepiece. This was not always easy due to the difraction pattern produced at the edge of the otolith under the optical microscope (recorded on the photographs).

We used the index of completion for current increment (C) established by Tanaka *et al.* (1981).

$$C = \frac{W_n}{W_n - 1} \times 100$$

where W_n = width of current increment.

$W_n - 1$ = width of previous complete increment.

The time of completion of a growth increment can thus be determined following the change of « C » in relation to the time of the day.

RESULTS

A total of 115 larvae were used in this study with total lengths comprised between 8 and 26 mm.

In the two previous papers (Ré, 1983a, 1983b) our data were consistent with the assumption that each otolith growth increment, described from the sagitta of pilchard larvae, represented one day. It was also shown from the statistical regression analyses, that otolith ring formation was probably initiated at a larval length of about 5.5 to 6 mm, the size of sardine larvae at the time of yolk sac absorption (3 to 5 days after hatching). Furthermore significant correlations were found between : larval length and otolith growth increments, sagitta length and otolith growth increments and larval length and sagitta length.

Again with the data obtained in the present study it was found that the correlations mentioned above were highly significant.

(i) *Larval length versus otolith growth increment linear regression equation* (Fig. 1).

$y = 1.773\,x - 9.175$ ($r = 0.936$, $n = 110$) growth rate 0,49 mm day $^{-1}$

where: x = total length of the larva

y = number of daily growth increments.

(ii) *Sagitta length versus otolith growth increment linear regression equation.*

$y = 0.235\,x + 3.675$ ($r = 0.940$, $n = 106$)

where: x = sagitta length

y = number of daily growth increments.

(ii) *Larval length versus sagitta length linear regression equation.*

$y = 7.222\,x - 49.307$ ($r = 0.959$, $n = 110$)

where: x = total length of the larva

y = diameter of sagitta.

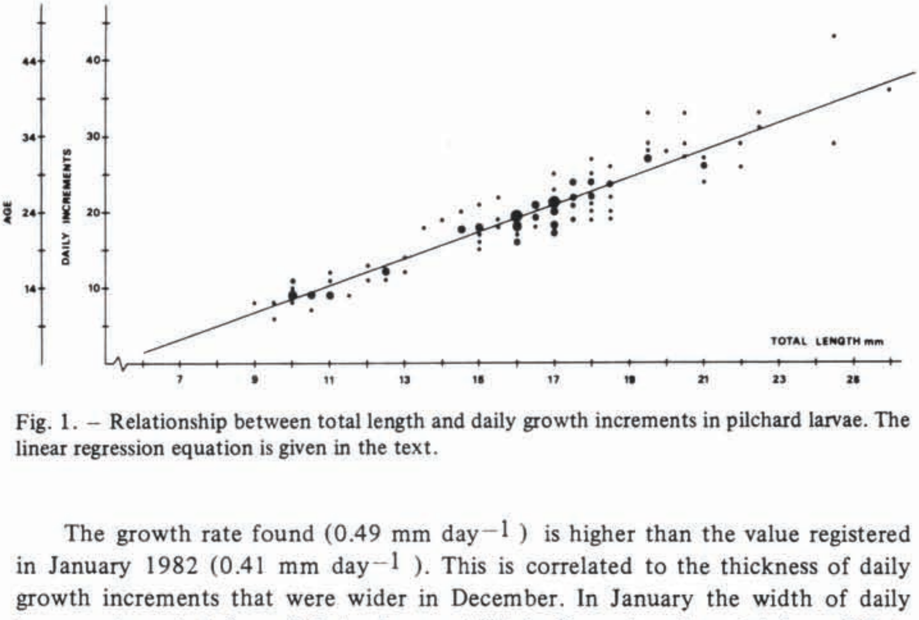

Fig. 1. – Relationship between total length and daily growth increments in pilchard larvae. The linear regression equation is given in the text.

The growth rate found (0.49 mm day^{-1}) is higher than the value registered in January 1982 (0.41 mm day^{-1}). This is correlated to the thickness of daily growth increments that were wider in December. In January the width of daily increments varied from 0.5 to 1 μm whilst in December it varied from 0.5 to 2.5 μm. A possible explanation for this fact might be the difference in food availability and different feeding conditions in the two periods (Ré, 1983b).

On a given otolith the width of growth units can vary significantly. Near the center of the otolith the first growth increments are normally smaller than the increments observed near the adge with some individual variations (wider increments may be found in certain areas of the otolith). Furthermore the nucleus had an average diameter of 13.0 μm ($s = 1.77$, $n = 50$).

The changes in the index of completion (Tanaka *et al.*, 1981) for current increments are represented in figure 2, for a period of 20 hours.

This index varied from 66.6 to 100 % between 15.30 h and 23.30 h and from 11.9 to 62.5 % between 00.30 h and 10.30 h (local time). The dark portion of the daily increment (organic, slow growth zone) visible under the optical microscope was formed during the dark period from 20.30 h to 23.30 h (first hours of darkness). The initiation of a new daily growth increment began around 00.30 h and corresponded to the deposition of a fast growth zone (inorganic).

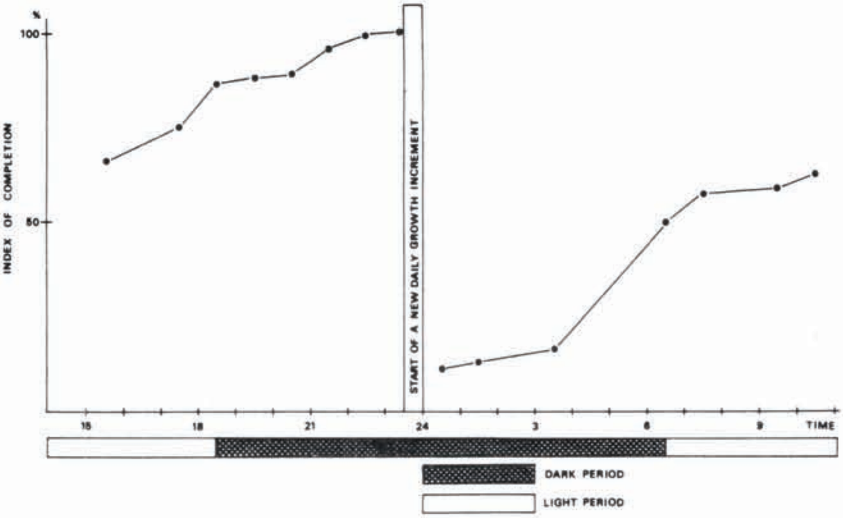

Fig. 2. – Changes in the index of completion for current increment (mean values) per hour during 20 hours in the otoliths of pilchard larvae.

The temperatures registered at the time of sampling remained almost constant during the 20 hours period (average surface temperature 15.59 ($s = 0.06$; average temperature at – 15 m 15.55 $s = 0.05$).

There seems to exist some relationship between thephotoperiod and the formation of the organic and inorganic portions of each daily growth unit. Nevertheless it is premature to relate any causative factor(s) with deposition of growth increments before we understand better the physiology of otolith formation.

Daily growth increments have been confirmed in a few fish species, however their occurence has been suggested in numerous other species. The method outlined in the present study provides an easy mean for corroborating the daily nature of otolith increments of field-captured larvae.

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